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Human Leukocyte Functions and the U.S. Safety Standard for Exposure to Radio-Frequency Radiation

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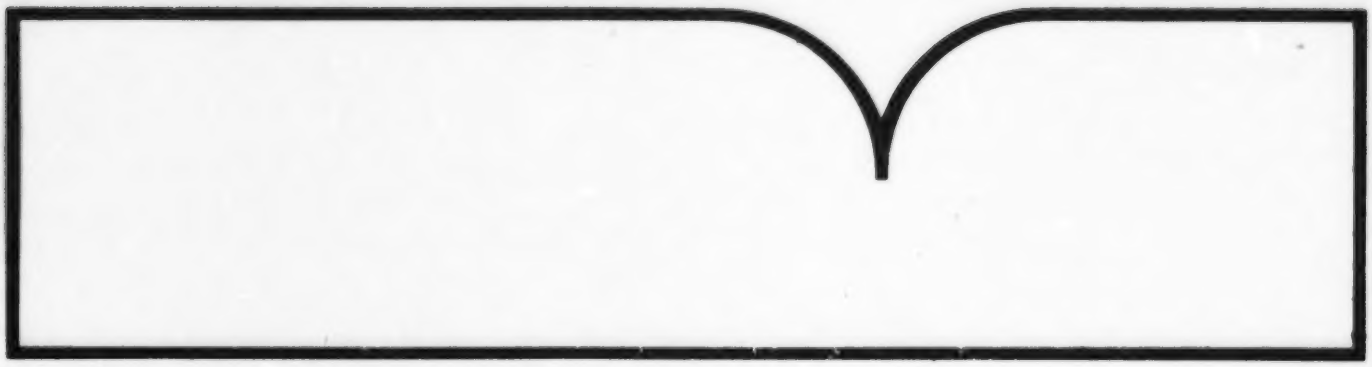

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16. ABSTRACT

Human mononuclear leukocytes were exposed to microwaves at energies relevant to current public safety recommedations. No detectable effects on viability or function of the leukocytes resulted from exposure to microwaves at specific absorption rates up to 4 milliwatts per milliliter. The results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate insofar as leukocyte function is concerned.

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a significant number of *S. aureus* strains from patients with TSS possess one or more common bacteriophage and that these phage can be transferred to another strain to yield a newly lysogenized bacterium. Another concept, namely that plasmids per se may be involved in TSS-associated strains, has been diminished by Kreiswirth *et al.* (*19*), who demonstrated a lack of association of plasmids with previously described toxin (or toxins) associated with TSS.

The question of whether or not lysogenic bacteriophage in TSS-associated strains of *S. aureus* are responsible for the production of pyrogenic exotoxin C/enterotoxin F, or perhaps a toxin or toxins not yet described, is unanswered. Blair and Carr (*14*) have already demonstrated that bacteriophage from an α-hemolysin–producing *S. aureus* strain could lysogenize another strain of *S. aureus* and that this was accompanied by a new capacity to produce α-hemolysin. Further experiments should show whether or not lysogenization of certain *S. aureus* strains with particular bacteriophage capable of inducing toxin formation plays the crucial role in initiating the pathological events that lead to toxic shock syndrome.

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Human Leukocyte Functions and the U.S. Safety Standard for Exposure to Radio-Frequency Radiation

Abstract. *Human mononuclear leukocytes were exposed to microwaves at energies relevant to current public safety recommendations. No detectable effects on viability or function of the leukocytes resulted from exposure to microwaves at specific absorption rates up to 4 milliwatts per milliliter. The results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate insofar as leukocyte function is concerned.*

All individuals are exposed to radio-frequency or microwave energies to variable degrees. Studies by several investigators have raised the possibility that the immunocompetent cells of humans are particularly susceptible to microwaves (*1*). These studies were admitted by at least some of the authors to be poorly reproducible and nonquantitative. Nonetheless, they are frequently cited, and they have provided the limited data available, on exposure of human leukocytes, for use by the individuals and agencies that develop environmental health standards (*2*). Many animal systems have been studied, but the species, microwave power intensities, environmental conditions, and other factors have been so varied that extrapolation to humans would be exceedingly difficult, even if appropriate (*3*, *4*).

The studies reported here provide data on exposure of human leukocytes to microwave energies relevant to current public safety recommendations. Exposure to microwaves at specific absorption rates up to 4 mW/ml resulted in no detectable effects on viability or on unstimulated or stimulated DNA, RNA, total protein, or interferon synthesis by human mononuclear leukocytes. In contrast to the studies cited above, the results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate.

Human mononuclear leukocytes were exposed in a waveguide system (*5*) to 2450-MHz (continuous-wave) microwaves for 2 hours at specific absorption rates (SAR's) of 0.5 to 4 mW/ml and were subsequently incubated in a humidified CO_2 incubator at 37°C. The safety standard limit proposed by Committee C-95.4 of the American National Standards Institute is 0.4 mW/g, which is equivalent in these cultures of 0.4 mW/ml. This standard incorporates approximately a tenfold safety factor relative to the basal metabolic rate for humans (3.5 mW/g). In addition to leukocyte cultures enclosed within waveguides for exposure or sham-exposure, we included control cultures located in the same incubator but external to the waveguides. No attempt was made to counteract microwave-induced heating of the leukocyte cultures since we wished to observe any potential microwave-induced effects, thermal or otherwise. Exposure of the leukocytes at an SAR of 4 mW/ml produced no significant changes in cell viability for up to 1 week after exposure (Table 1); results were similar with exposures at lower SAR's.

Unstimulated and mitogen-stimulated DNA, RNA, and total protein synthesis was examined after exposure of the mononuclear leukocytes to microwaves at SAR's of 4 mW/ml or less. There were no significant differences between microwave-exposed (4 mW/ml), sham-exposed, and control leukocytes in unstimulated nucleotide or protein synthesis, or in responses of the leukocytes to an optimal concentration of mitogen (Fig. 1). Similar results were obtained with suboptimal concentrations of mitogen and lower SAR's (0.5 and 1.0 mW/ml) for the microwave-exposed cultures (data not shown). Microscopic inspection of Wright-Giemsa–stained cytospin preparations (*6*) did not reveal any discrepancies between morphologic lymphocyte blastogenesis (used in some of the studies cited earlier) and determinations based on incorporation of the radiolabeled precursors.

Table 1. Total viable mononuclear leukocytes after exposure to microwave energy at an SAR of 4 mW/ml. Each value is the mean total number of viable cells (total cells × percent viable) × 10^{-4}, ± standard error. Insufficient numbers of observations (< 5) were available 3 days after exposure. Viability was assessed by the ability of the cells to exclude trypan blue dye and ethidium bromide.

Days after exposure	Mean total number of viable cells		
	Microwave	Sham	Control
1	58 ± 7	65 ± 11	54 ± 6
2	60 ± 9	63 ± 15	56 ± 6
4	41 ± 4	39 ± 2	41 ± 8
5	47 ± 16	72 ± 36	46 ± 13
6	39 ± 10	41 ± 9	36 ± 7
7	40 ± 10	37 ± 8	38 ± 11

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In addition to total protein synthesis, we measured spontaneous production of interferon (none detected in any cultures) and production of influenza virus–induced α-interferon and phytohemagglutinin (PHA)-induced γ-interferon at 1 and 3 days after induction (*7*). Virtually all detectable virus-induced α-interferon was present by 24 hours, with equivalent amounts produced by microwave-exposed (SAR, 4 mW/ml), sham-exposed, and control leukocytes (9.59 ± 0.58, 9.61 ± 0.70, and 9.33 ± 0.67 $\log_2$ units per milliliter, respectively). Phytohemagglutinin-induced γ-interferon, usually produced by 48 to 72 hours, was not detected in any culture supernatant fluid by 24 hours. By 72 hours, γ-interferon was detected in all PHA-induced cultures, with no significant differences between the microwave-exposed (SAR, 4 mW/ml), sham-exposed, and control leukocytes (6.82 ± 0.71, 7.62 ± 1.09, and 6.99 ± 0.33 $\log_2$ units per milliliter, respectively). Similar results were obtained with exposures to microwaves at lower SAR's.

These studies provide clear, reproducible data regarding exposure of human leukocytes to microwave energies relevant to current public safety recommendations. Direct extrapolation to the in vivo setting with many physiological, homeostatic, integrated systems is not appropriate. However, these data suggest that earlier reports of possible microwave effects on human leukocytes, at such energy levels, should not form a basis for the resetting of safety standards. Most studies of environmental physical factors examine effects on resting cell populations even though, under normal conditions, humans are commonly exposed to more than one environmental stress at a time (*8*). Thus, these results further indicate that human leukocytes exposed to microwaves, as a potential physical stress factor, can respond normally to a second biological factor, such as the commonly encountered infectious agent influenza virus.

These studies do not exclude the possibility of microwave-induced effects on human leukocytes resulting from exposures at greater SAR's. Such exposures commonly produce effects that can be related to the degree or the rate of heating of the cell cultures or tissues in vivo (*9*). Also not completely excluded are potential microwave-induced effects resulting from exposure at similar SAR's but different wave forms (frequencies, modulations, and so on).

The ubiquitous distribution of microwave energy and the potential differences between animal models and humans suggest that further investigations with human leukocytes and other cells may be warranted. The literature on microwaves includes animal studies showing deleterious effects of exposure and animal studies showing beneficial effects, over a broad range of SAR's. Potential health hazards for humans should be further defined and limited, and potential health benefits, such as the use of microwave-induced hyperthermia in the treatment of cancer (*10*), should be further defined and expanded.

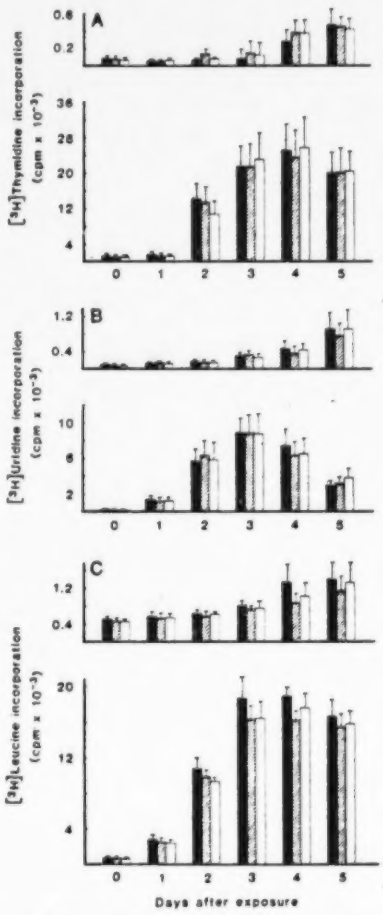

Fig. 1. DNA, RNA, and total protein synthesis by (■) microwave-exposed, (▩) sham-exposed, and (□) unexposed (control) human mononuclear leukocytes. Mononuclear leukocytes were obtained by Ficoll-Hypaque separation and exposed (4 mW/ml) or sham-exposed to microwaves. Control leukocytes were cultured in the same incubator but external to the waveguides. DNA, RNA, and total protein synthesis were measured by cellular incorporation of tritiated thymidine (A), uridine (B), and leucine (C), respectively, using established methods (*11*). Unstimulated and mitogen-stimulated precursor incorporation (top and bottom histograms in each panel, respectively) were assayed from immediately to 5 days after exposure. The mitogen phytohemagglutinin was added at a concentration of 160 μg/ml, which yields optimum responses with normal mononuclear leukocytes, as well as at several suboptimal concentrations (20 to 80 μg/ml). Responses (mean counts per minute ± standard error) are shown for unstimulated and optimal phytohemagglutinin-stimulated leukocytes.

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19 August 1982; revised 20 December 1982

Sodium Currents in Segments of Human Heart Cells

Abstract. *Isolated human heart cells were partially drawn into the lumen of a plastic tube and cleaved at the partitioning tube wall by intraluminal suction pulses. The extraluminal segment (10 to 20 percent of the cell length) was suitable for intracellular perfusion and voltage clamp. The time and voltage dependence of the sodium current, and the responses to changes in driving force and channel blockers, illustrate the potential of these preparations as models for the study of membrane channels.*

The complex structure and large membrane area of multicellular cardiac preparations preclude or greatly complicate the measurement of sodium current (I_{Na}) in the heart (*1*). A recent solution has been to apply voltage clamp techniques to single cells isolated from rat cardiac tissue (*2*, *3*). By employing a modification of a cell dialysis–voltage clamp technique (*3*, *4*), we measured I_{Na} in single myocytes isolated from human heart tissue.

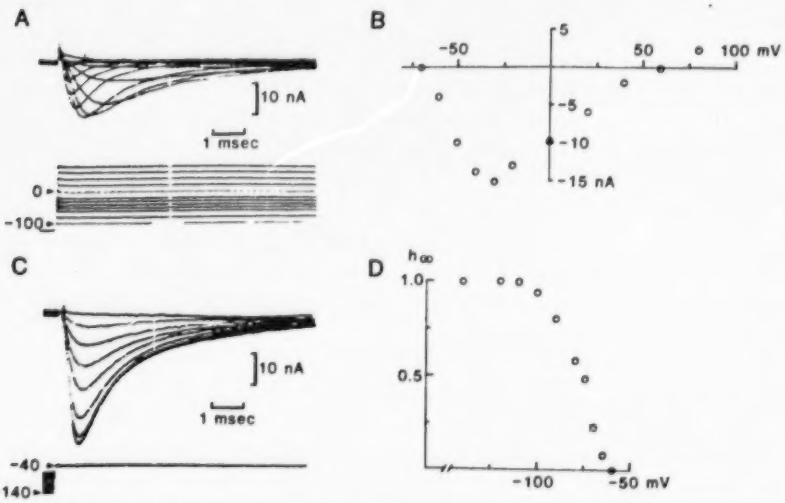

Fig. 1. Sodium current in human atrial cell segments. Pulsing rate was 0.2 Hz. (A) Currents accompanying 10-msec-long depolarizations from −120 mV. (B) Current-voltage relation of peak I_{Na} from the records shown in (A). Peak I_{Na} was measured with reference to zero current. (C) I_{Na} in a different cell. Test pulses to −40 mV were preceded by 100-msec pulses from −120 mV to potentials between −140 and −60 mV. (D) Steady-state inactivation of I_{Na} measured from the records in (C).

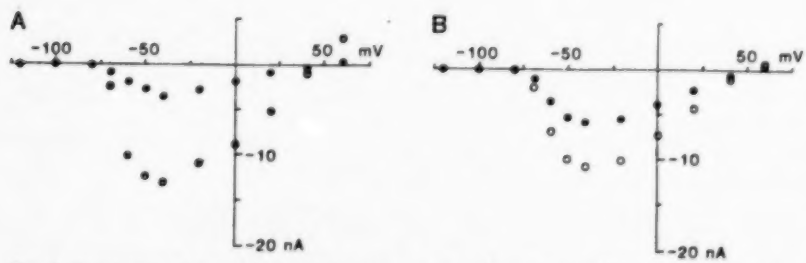

Fig. 2. Depression of I_{Na} after 5-minute exposure to sodium channel blockers. Experimental protocol was similar to that in Fig. 1, A and B. (○) Controls; (●) 5 μ*M* TTX in (A) and 250 μ*M* lidocaine in (B).

Cells were isolated from nondiseased atrial specimens obtained during corrective cardiac surgery (*5*). They were collected during the first 30 to 45 minutes of tissue digestion with collagenase V (300 U/ml; Sigma) and protease VII (1.0 U/ml; Sigma) in calcium-free solution. The calcium-free solution contained (millimoles per liter): 150.0 NaCl, 2.5 CsCl, 2.5 Cs-Hepes, 2.5 Hepes–free acid, 1.0 $MgCl_2$, and 10.0 glucose (pO_2 100 to 150 mmHg, *p*H 7.3). The isolated cardiocytes are about 100 μm long and 10 μm wide, have a morphology similar to that of cells dispersed from laboratory animal hearts (*6*), and display simultaneous action potentials and contractions after electrical stimulation (*5*).

The apparatus for intracellular perfusion and voltage clamp was similar to that described by Kostyuk *et al.* (*4*). A pore 2 to 4 μm narrower than the width of the cell to be studied was bored through the 4- to 6-μm wall of the V-shaped plastic tube. Pore resistance was 100 to 200 kilohms and accounted for most of the system series resistance. Series resistance compensation was achieved by conventional means (*4*).

The cell and tube were viewed through the glass bottom of a 1-ml bath with a modified, inverted microscope at ×100 to ×800 magnification. After the pore was positioned near one end of the cell, a small negative hydrostatic pressure of 5 to 10 mmHg was applied to the interior of the tube to attract the cell to the pore. An increase in negative pressure resulted in the aspiration of 80 to 90 percent of the cell into the lumen of the tube, and a series of suction pulses (about 400 mmHg, 1 second) sheared off the intraluminal cell segment.

The intracellular perfusate flowing in the tube contained (millimoles per liter): 150.0 CsF, 10.0 NaF, 2.5 Cs-Hepes, and 2.5 Hepes–free acid (*7*). The bath was perfused with calcium-free solution for 5 minutes before and during and for 5 minutes after cell aspiration into the tube; this prevented suction-induced contracture. Recovery of the test segment was then facilitated by addition of 1.0 m*M* $CaCl_2$ to the external solution and by application of hyperpolarizing currents (1 to 30 nA). After 1 to 3 minutes, a current of less than 1 nA was sufficient to maintain a stable membrane potential (V_m) of −120 mV.

Experiments were performed at 20° to 22°C and the preparation was usually stable for 30 to 90 minutes. To suppress calcium current, $MnCl_2$ (3 m*M*) was added to the external solution. Electrical signals were filtered at 10 kHz, displayed on a storage oscilloscope (Tektronix